PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Taylor *et al.*

Serial No.: 09/386,605

Filed: August 31, 1999

FOR: NOVEL TRANSGENE ASSAY USING STABLE *AGROBACTERIUM RHIZOGENES* TRANSFORMATION

Group Art Unit: 1638

Examiner: Page, Brent T.

Atty. Dkt. No.: 11000023-2230 MONS:131US

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October 20, 2008 — Date

/ Robert E. Hanson/ — Robert E. Hanson

REPLY BRIEF

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BRIEF ON APPEAL

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Appellants hereby submit this Reply Brief responsive to the Examiner's Answer dated August 18, 2008. The date for filing this Brief is Monday, October 20, 2008. No fees are believed due. However, should any fees become due under 37 C.F.R. §§ 1.16 to 1.21 for any reason relating to the enclosed materials, or should an overpayment be made, the Commissioner is authorized to deduct or credit said fees from or to Sonnenschein Nath & Rosenthal LLP Deposit Account No. 19-3140/MONS:131US.

I. REAL PARTY IN INTEREST

The real party in interest is Monsanto Company, the parent company of assignee Monsanto Technology LLC.

II. RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences.

III. STATUS OF THE CLAIMS

Claims 1-26 were filed with the application. Claims 2-7 were canceled and claims 12-26 are withdrawn as being directed to a non-elected restriction group. Claims 1 and 8-11 are therefore currently pending and under examination. Claims 1 and 8-11 were rejected by the Examiner in the Final Action dated October 18, 2007 and are the subject of this appeal. A copy of the appealed claims as they currently stand is provided in Section VIII.

IV. STATUS OF AMENDMENTS

An amendment to claims 1, 8 and 11 was made in the Response to Office Action filed on June 11, 2002 and was entered by the Examiner. An amendment to claim 1 was made in the Response to Office Action filed on December 2, 2003 and was entered by the Examiner. An amendment to claims 1 and 8 was made in the Response to Office Action filed on April 11, 2005 and was entered by the Examiner. An amendment to claim 1 was made in the Response to Office Action filed on April 20, 2006 and was entered by the Examiner. An amendment to claims 1 and 8 was made in the Response to Office Action filed on November 20, 2006 and was entered by the Examiner. An amendment to claim 1 was made in the Response to Office Action filed on July 27, 2007 and was entered by the Examiner. No subsequent amendments have been filed.

V. SUMMARY OF CLAIMED SUBJECT MATTER

Independent claim 1 relates to a method for producing a stably transformed chimeric dicotyledonous plant having transgenic root tissue. Specification from page 3, line 10 to page 3, line 12. The method comprises the steps of:

obtaining a stem or hypocotyl explant from a selected dicotyledonous plant species, wherein the hypocotyl explant has a cut end below the cotyledon (Specification from page 7, line 15 to page 7, line 17);

transforming the stem or hypocotyl explant with *Agrobacterium rhizogenes* containing an exogenous nucleic acid sequence capable of being transferred to the explant, wherein the cut end of the hypocotyl explant is contacted with the *Agrobacterium rhizogenes* (Specification from page 7, line 19 to page 7, line 21);

culturing the transformed explant in a root initiating media to produce transformed roots (Specification from page 7, line 27 to page 7, line 28); and

transferring the transformed roots to soil or a hydroponic environment to produce a chimeric dicotyledonous plant having transformed roots and wild type shoots, stems and leaves (Specification from page 7, line 31 to page 7, line 34), wherein the dicotyledonous plant is soybean (Specification from page 8, line 20 to page 10, line 31).

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Were claims 1 and 8-11 properly rejected under 35 U.S.C. 103(a) as being unpatentable over Trulson *et al.* (EP 0262972 A2) in view of Simpson *et al.* (1986 *Plant Mol. Biol.* 6:403-415) and further in view of Savka *et al.* (1990 *Phytopathology* 80:503-508)?

VII. REPLY

A. The Answer Fails to Rebut Appellants Showing That All Elements Of The Claims Are Not In The Prior Art

In Appellants Brief, it was shown that none of the cited references, or the art generally, teach or suggest the element in claim 1 of a "chimeric dicotyledonous plant having transformed roots and wild type shoots, stems and leaves…" For example, in asserting obviousness the Examiner alleged that Trulson *et al.* ("Trulson") teaches a method of producing a stable transformed chimeric cucumber plant having transgenic root tissue, and that Trulson demonstrate chimeric cucumber plants at page 6, line 45-55, describing 690 roots from *A. rhizogenes*-inoculated hypocotyl sections, where 64 roots regenerated plants, 22 of which were positive for NPTII, the transgenic selection marker. Of 11 plants that had not been selected for NPTII expression (Series A), two were said to be positive for NPTII, whereas the remaining 9 did not have the selectable marker. The Examiner asserts that some or all of these 11 plants are chimeric. This assertion is not consistent with the teachings of Trulson.

1. Trulson Does Not Teach Chimeric Mother Plants

The Examiner's Answer asserts that the mother plant (hypocotyl + stem + other green parts) of each of the two NPTII-positive plants was chimeric "since only newly growing roots would have been transformed." Examiner's Answer, page 5 bottom. This is incorrect.

The 11 Series A plants were created by inoculating cucumber hypocotyl sections with *A. rhizogenes* then, after one week, transferring the inoculated sections into media without kanamycin. Trulson at p. 5, lines 52-62. The roots produced on the inoculated surfaces of the inoculated sections were excised and placed on media without kanamycin. After 2-3 weeks, embryoids that appeared on the root surface were detached and transferred to another media without kanamycin for 10-14 days to develop mature embryoids. *Id.* at p. 6, lines 9-11. The

mature embryoids were transferred to still another media without kanamycin where plantlets (with shoots) were produced.

As can be seen from the above description, there was no "mother plant" that gave rise to the two plants that were NPTII positive, since roots that were produced on the inoculated surfaces were not grown to full plants, but instead embryoids that appeared on the surface were excised and grown into full plants. Additionally, none of the roots of the 11 Series A plants (or any other plants described in Trulson) were tested for NPTII. Thus, the two plants whose leaves were NPTII-positive likely had roots that were NPTII positive; similarly, the 9 plants whose leaves were NPTII-negative likely had roots that were NPTII-negative. There is no evidence whatsoever that any of the 11 Series A plants were chimeric. Indeed, Trulson *et al.* never tested any roots for NPTII or any other indicia of transformation, and never suggested that any plants from that study were chimeric.

2. The Answer's Argument That Cefotaxime is a Selection Agent is Incorrect

The Answer also asserts that the 9 unselected Series A plants that did not have NPTII in the shoots are chimeric, even though the Examiner stated earlier in the Answer that only the mother plants of the two Series A plants that tested positive were chimeric. Answer at p.8. In making the argument that the 9 unselected Series A plants are chimeric, the Examiner newly asserts that the 9 plants must be chimeric because they were selected on cefotaxime, which "was used to ensure that recovered cells contained the transformation vector." Answer at page 8. The Examiner makes the same argument in asserting that the 33 NPT-negative plantlets in the Series B (selection) experiments must be chimeric, asserting that, even if non-transgenic plants can escape kanamycin selection, they could not escape cefotaxime selection. Answer at page 9. Thus, the Examiner argues that cefotaxime was actually a selection agent that forced the roots of

the 9 Series A plants and the 33 NPTII-negative Series B plants to retain the vector. This is incorrect.

Appellants note that Trulson states at page 5, lines 61-62, that "Cefatoxime, an ampicillin analogue, was used since it is not modified by the beta-lactamase encoded by the Amp^R gene on the vectors pARC8 or pARC16." Trulson *et al.* also state, at page 6, lines 2-3, "To eliminate bacterial carry-over, the media contained 100mg/l of cefotaxime." Thus, cefotaxime was clearly used in Trulson *et al.* as an ***agent to kill the Agrobacterium rhizogenes inoculum***; there is no indication in Trulson *et al.* that cefotaxime was used as a ***plant selection agent***.

The scientific literature of record in this case confirms that cefotaxime is used to kill *Agrobacterium* inoculum and is ***not a selection agent in plant transformation***. See, *e.g.*, Simpson *et al.* (cited in the current rejection), stating at page 407, left column,

> "The stems were cut in sections (2-3 cm), inverted and transferred aseptically to solid hormone-free TM-1 medium (40) + 250 mg/l cefoxitin (Merck, Sharp and Dohme) or recently, cefotaxime (Calbiochem) in plastic boxes. Some of the *Agrobacterium* strains are resistant to ampicillin and to Carbenicillin but not cefoxitin (11) or cefotaxime (23)."

Also, Rech *et al.*, 1989, Plant Cell Reports 8:33-36 (cited by the Examiner on November 29, 2001) states on page 33, right column,

> "Roots produced at inoculation sites . . . were excised and transferred to 9 cm Petri dishes . . . each containing 20 ml of B50 medium with 300 $\mu g ml^{-1}$ cefotaxime . . . and 50 $\mu g ml^{-1}$ of kanamycin sulphate (Sigma). . . . Root clones were established by excising single root tips . . . and subculturing to fresh medium every 14 days. After 5 subcultures, the roots were maintained on B50 medium lacking cefotaxime, but with kanamycin (50-100 $\mu g ml^{-1}$)."

Since Rech *et al.* used kanamycin without cefotaxime after several subcultures, cefotaxime is clearly not used for selection, since it was not used when selection was applied with kanamycin.

Simpson *et al.* and Rech *et al.* clearly indicate that cefotaxime is not considered to be a selectable agent for plants. In light of the above, Appellants assert that cefotaxime is not a

selective agent, as claimed by the Examiner. Since cefotaxime is not a selective agent, there remains no reason to believe that the 9 NPTII-negative Series A plants and the 33 NPTII-negative Series B plants have transformed roots, and the rejection remains unsupported.

3. The Lack of Support for the Rejection is Demonstrated by Inconsistencies in Assertions by the Examiner Regarding Which Plants of Trulson Are Chimeric

Contrasting the assertion in the Examiner's Answer that the mother plants of the two NPTII-positive Series A plants are chimeric, the Examiner asserted in the Final Office Action dated October 18, 2007 that the 9 plantlets that were ***not*** positive for NPTII were chimeric:

> However, the results show that out of 11 plantlets regenerated only 2 were NPT-positive, indicating that 9 out of 11 plantlets did not contain the selectable marker in the plantlets in sufficient quantity to be NPT positive. Therefore, Trulson teaches transgenic root tissue with non-transgenic shoots.

Final Office Action of October 18, 2007 at page 3. In further contrast, the Examiner asserted in the Nonfinal Office Action dated March 27, 2007 that all 11 Series A plants were chimeric:

> The plantlets of Series A above, which were produced without selection on kanamycin, contained some transgenic tissue and some wild-type tissue. Since Agrobacterium rhizogenes transformation produces transgenic root tissue, some of the roots produced are transgenic and some are not. Accordingly, Series A plantlets, not raised under selection, will be a population of plantlets having some transgenic roots and some wild-type shoots, stem and leaves. Also present in the Series A plantlets are those having transgenic roots, and a mixture of transgenic and wild-type shoots, stem and leaves. These plants are chimeric cucumber plants having some transgenic roots and some wild-type shoots, stem and leaves, as set forth in claim 1.

Nonfinal Office Action of March 27 2007 at pages 5-6. Thus, the Examiner has changed arguments from the Nonfinal Office Action to the Final Office Action and to the Examiner's Answer, apparently changing the rationale explaining which of the 11 plants are chimeric in response to Appellants' arguments against the various rationales. Indeed, in the Examiner's Answer, the Examiner does not assert that ***any*** of the 11 plants are chimeric, but instead the

mother plants of the two NPTII-positive plants were chimeric. These changes in position demonstrate that none of the arguments made are supportable by the facts of record.

In response to Appellants' arguments in the Appeal Brief (starting at page 7 of the Examiner's Answer), the Examiner further points to Table I on page 7 of Trulson, stating that the first line of Table I shows two chimeric plants arising from the Series A study, where there was no selection on kanamycin. However, as discussed above, the Examiner asserted earlier in the Answer that these two plants were ***not*** chimeric, but the mother plants that gave rise to these plants was chimeric (although the discussion above establishes that there was no mother plant that gave rise to these plants). The Examiner also states in the Answer that

> the second line shows that even in the presences of the selection agent of kanamycin there are 33 NPT-negative plantlets indicating that the roots, being the tissue used for regeneration necessarily contain the construct in order to survive the media with kanamycin and that the leaves, being NPT-negative, do not contain the construct. This is evidence that chimeric plants have been generated, and further, that these plants are chimeric plants that have transgenic roots and wild-type (NPT-negative) shoots, stems and leaves.

Examiner's Answer at page 7-8.

However, the Examiner again failed to consider that the 33 NPT-negative plantlets could have simply been non-transgenic escapes that grew in spite of the kanamycin selection. Indeed, Trulson *et al.* express a belief that these plants are not transformed, at page 7, lines 25-27, by stating, "[t]he addition of 25 mg/l kanamycin did not affect the regeneration process of the transformed tissue, ***nor did it prevent regeneration of some NPT-negative plants***." (emphasis added). In response to this statement, the Examiner asserts that the statement does not count as an argument that non-transgenic plants escaped kanamycin selection because Trulson *et al.* was interested in regenerating transgenic plants and would discard plants that had NPTII-negative leaves without determining whether the roots were transformed or not. However, Appellants

point out that this does not provide any evidence that the roots ***were*** transformed in those plants, and Trulson's statement is consistent with other literature that establishes that kanamycin selection regimes yield some non-transgenic plants. This escape from selection can occur, for example, by transient, non-stable transgenic expression where a transgene does not stably insert into the genome. Again, Trulson did not test the roots for NPT so there is no evidence that any of the plants were chimeras; Trulson simply does not make any such suggestion.

The Examiner further states that "the generation of such chimeric plants is a normal part of transformation methods and procedures." Examiner's Answer, page 8. However, no evidence is provided that ***stable*** chimeric plants, with transformed roots and wild-type shoots, stems and leaves, as claimed, have been produced by normal transformation methods and procedures.

To summarize the above, there is no evidence in Trulson that stable chimeric plants were produced, since the data disclosed therein is entirely consistent with the assertion that the plants that did not have NPTII in their shoots likely also did not have NPTII in their roots, *i.e.*, they were not transformed at all.

B. Simpson *et al.* and Savka *et al.* Do Not Teach or Suggest Claim Elements Lacking in Trulson

To establish a *prima facie* case of obviousness, the prior art references or art generally must teach or suggest all the claim limitations. M.P.E.P § 706.02(j).

The Examiner cites Simpson *et al.* and Savka *et al.* as teaching soybean plant systems which may be transformed using *A. rhizogenes*. However, because Trulson *et al.* do not teach a stably transformed chimeric dicotyledonous plant with transformed roots and wild type shoots, stems and leaves, as thoroughly explained above, the claims can only be obvious if Simpson *et al.* and Savka *et al.* teach or suggest this missing element of Applicants' invention. As discussed

in the Appeal Brief and acknowledged by the Examiner, Simpson *et al.* and Savka *et al.* do not teach a chimeric plant.

C. Conclusion

In light of the above, Applicants respectfully submit that no combination of the cited references teach or suggest the production of a stably transformed chimeric dicotyledonous plant having transgenic root tissue and wild type shoots, stems and leaves. Therefore Applicants' invention is not rendered obvious from them and reversal of the rejection is therefore respectfully requested.

It is also respectfully submitted, in light of the above, that none of the claims are properly rejected. Therefore, Appellants request that the Board reverse the pending grounds for rejection.

Respectfully submitted,

/ Robert E. Hanson/

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Date: October 20, 2008

VIII. CLAIMS APPENDIX

APPEALED CLAIMS:

1. A method for producing a stably transformed chimeric dicotyledonous plant having transgenic root tissue, the method comprising the steps of:

obtaining a stem or hypocotyl explant from a selected dicotyledonous plant species, wherein the hypocotyl explant has a cut end below the cotyledon;

transforming the stem or hypocotyl explant with *Agrobacterium rhizogenes* containing an exogenous nucleic acid sequence capable of being transferred to the explant, wherein the cut end of the hypocotyl explant is contacted with the *Agrobacterium rhizogenes*;

culturing the transformed explant in a root initiating media to produce transformed roots; and

transferring the transformed roots to soil or a hydroponic environment to produce a chimeric dicotyledonous plant having transformed roots and wild type shoots, stems and leaves, wherein the dicotyledonous plant is soybean.

8. The method of claim 1 wherein transformed roots are initiated in the hypocotyl by placing the end of the hypocotyl contacted with the *Agrobacterium rhizogenes* in a media containing ¼ strength Murashige and Skoog media.

9. The method of claim 8 wherein the media further comprises a selectable agent.

10. The method of claim 9 wherein the selectable agent is kanamycin.

11. The method of claim 10 wherein the concentration of kanamycin in the media is no more than 50 mg/L.

IX. EVIDENCE APPENDIX

Exhibit A: Trulson *et al.*, "Genetic transformation and controlled regeneration of cucumis SP in vitro." European Patent Publication No. 0262972, published June 4, 1988. Cited by Examiner. (Copy submitted with Appellants' Appeal Brief)

Exhibit B: Simpson *et al.*, "A disarmed binary vector from *Agrobacterium tumefaciens* functions in *Agrobacterium rhizogenes*," *Plant Molecular Biology* 6: 403-415, 1986. Cited by Examiner. (Copy submitted with Appellants' Appeal Brief)

Exhibit C: Savka *et al.*, "Induction of hairy roots on cultivated soybean genotypes and their use to propagate the soybean cyst nematode," *Phytopathology* 80: 503-508, 1990. Cited by Examiner. (Copy submitted with Appellants' Appeal Brief)

X. RELATED PROCEEDINGS APPENDIX

There are no related proceedings.